

Drawings:

Please delete figures 19A and B.

REMARKS

The specification is amended to correct the omission of the sequence identification numbers for SEQ ID NOs 1-22 in the specification and the omission of the brief description of figure 18. Marked up versions of the amended paragraphs are included in Attachment A. The sequence identification numbers are the same as those in the sequence listing in paper and computer readable form. No new matter is introduced. In addition, Applicants would like to amend the drawings to delete figure 19A and B.

The Commissioner is hereby authorized to charge any fees associated with this paper to Deposit Account No. 01-0431.

Respectfully submitted,

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Attachment A: Marked up paragraphs according to 1.121(b)(iii):

Paragraph beginning on page 12, line 23:

The Type-IIs endonucleases, on the other hand, generally do not require palindromic recognition sequences. Additionally, these Type-IIs endonucleases also generally cleave outside of their recognition sites. For example, the Type-IIs endonuclease EarI recognizes and cleaves in the following manner:

C T C T T C N \downarrow N N N N (SEQ ID NO: 1)

G A G A A G n n n n \downarrow n (SEQ ID NO: 2)

where the recognition sequence is -C-T-C-T-T-C-, N and n represent complementary, ambiguous base pairs and the arrows indicate the cleavage sites in each strand. As the example illustrates, the recognition sequence is non-palindromic, and the cleavage occurs outside of that recognition site.

Paragraph beginning on page 25, line 3:

Adaptor sequences containing PCR primer template sequences were then ligated to the purified fragments using 100U T4 ligase in 1x T4 DNA ligase buffer (New England Biolabs) at 16 °C overnight. The adaptor sequences were 5'-d(pAATTCTGAACCCCTTCGGATC)-3' (SEQ ID NO: 3) and 5'-d(GATCCGAAGGGGTTCGAATT)-3' (SEQ ID NO: 4) (Figure 2, Step 4) The ligase was then heat inactivated at 65 °C for 15 minutes.

Paragraph beginning on page 25, line 8:

The fragments were then subjected to PCR with one primer that corresponded to the PCR primer template sequence 5'-d(GATCCGAAGGGGTTCGAATT)-3' (SEQ ID NO: 5) (Figure 2, Step 5). The PCR mixture contained approx. 1 ng ligated DNA fragments, 5 units AmpliTaq Gold polymerase (Perkins Elmer), 5 uM pimer, 200uM dNTPs, 15 mM Tris-HCl (pH8.2), 50 mM KCl, 2.5 mM MgCl₂ in a final volume of 50 ul. PCR was performed in a Perkin-Elmer 9600 thermocycler using an initial 10 minute denaturation at 95 °C, 35 cycles of a 1 minute denaturation at 94 °C, annealing for 1 minute at 57 °C and extension at 72 °C for 2 minutes. This is followed by a final 5 minute extension cycle at 72 °C.

Paragraph beginning on page 26, line 22:

Adaptors containing PCR primer template sequences were ligated in a 50 ul mixture of 400 ng digested genomic DNA, 10 pmol adaptor and 40 unit T4 ligase in a 1X T4 ligase buffer. (Figure 3, Step 2) The adaptor sequences were as follows: 5'-d(pATNNGATCCGAAGGGTTCGAATTC)-3' (SEQ ID NO: 6) and 5'(GAATTCGAACCCCTTCGGATC)-3' (SEQ ID NO: 7). The ligation was conducted at 16°C overnight. The ligase was inactivated by incubation at 65°C for 15 minutes.

Paragraph beginning on page 26, line 28:

The fragments were then subjected to PCR with one primer that corresponded to the PCR primer template sequence: 5'-(GAATTCGAACCCCTTCGGATC)-3' (SEQ ID NO: 8) in a 50 ul reaction containing 20 ng ligated DNA, 1 unit AmpliTaq Gold polymerase (Perkins Elmer), 3 uM primer, 200uM dNTPs, 15 mM Tris-HCl (pH8.0), 50 mM KCl, 2.5 mM MgCl₂. PCR was performed in a Perkin-Elmer 9600 thermocycler using an initial 10 minute denaturation at 95°C, 35 cycles of a 0.5 minute denaturation at 94°C, annealing for 0.5 minute at 57°C and extension at 72°C for 2 minutes. This is followed by a final 5 minute extension cycle at 72°C.

Paragraph beginning on page 27, line 14:

The restriction fragments were then ligated to adaptor sequences. The ligation mixture contained: 5 pmol Eco R I adaptor [5'-d(pAATTCGAACCCCTTCGGATC)-3' (SEQ ID NO: 9) and 5'-d(GATCCGAAGGGGTTTCG)-3' (SEQ ID NO: 10)], 50 pmol Sau3A I adaptor [5'-d(pGATCGCCCTATAGTGAGTCGTATTACAGTGGACCATCGAGGGTCA)-3' (SEQ ID NO: 11)], 5 mM DTT, 0.5 ng/ul BSA, 100 unit T4 DNA ligase, 1 mM ATP, 10 mM Tris-Acetate (pH 7.5), 10 mM magnesium acetate and 50 mM potassium acetate]. The ligation mixture was incubated with the restriction fragments at 37°C for 3 hours. The ligase was inactivated at 65 °C for 20 minutes.

Paragraph beginning on page 27, line 22:

The ligated DNA target was then amplified by PCR. The PCR mixture contained 12.5 ng ligated DNA, 1 unit AmpliTaq Gold polumerase (Perkins Elmer), 0.272 mM EcoRI selective primer (5'-AAGGGGTTTCGGAATTC-3'; (SEQ ID NO: 12) CC as the selective bases), 0.272 uM Sau3AI selective primer (5'-TCACTATAGGGCGATCTG-3'; (SEQ ID NO: 13) TG as the selective bases), 200 uM dNTPs, 15 mM Tris-HCl (pH 8,0), 50 mM KCl, 2.5 mM MgCl₂ in a final volume of 50 ul. PCR was performed in a Perkin-Elmer 9600 thermocycler using an initial 10 minute denaturation at 95 °C, 35 cycles of a 1 minute denaturation at 94 °C, annealing for 1 minute at 56 °C and extension at 72 for 2 minutes. This is followed by a final 5 minute extension at 72 °C.

Paragraph beginning on page 30, line 14:

Adaptor sequences containing PCR primer templates were then ligated to the DNA sequences in a 10 ul ligation mixture: 1ul DNA solution, 4 ul dH₂O, 1 ul 10X T4 DNA ligase buffer, 3 ul 10 mM adaptor [5'-d(GATCCGAAGGGGTTTCGAATT)-3' (SEQ ID NO: 14) and 5'-d(pGAATTCGAACCCCTTCGGATC-3')

(SEQ ID NO: 15) and 1 ul 400 U/ul T4 DNA ligase] and incubated at 16 °C overnight and then inactivated at 65 °C for 15 minutes. (Figure 7, Step 4)

Paragraph beginning on page 30, line 20:

The sequences were amplified in a 25 ul reaction containing 0.25 pmol template DNA, 0.125 units AmpliTaq Gold polymerase (Perkin Elmer), 3 uM primer, [5'-d(GATCCGAAGGGGTTCTGAATT)-3' (SEQ ID NO: 16)], 200 uM dNTPs, 15 mM tris-HCl (pH 8.0), 50 mM KCl and 1.5 mM MgCl₂.

Paragraph beginning on page 31, line 16:

The beads were then resuspended in ligation mixture containing T4 ligase in 1 X T4 ligase buffer and 200 fold excess adaptor I sequence [5'-d(ATTAAACCCTCACTAAAGCTGGAG)-3' (SEQ ID NO: 17) and 5'-d(pCTCCAGCTTTAGTGAGGGTTAAT)-3' (SEQ ID NO: 18) BpmI recognition sites are highlighted in boldface] at 16 °C overnight. The ligase was then inactivated by incubation at 65 °C for 10 minutes.

Paragraph beginning on page 31, line 27:

A second set of adaptor sequences containing PCR template sequences [5'-d(pCTATAGTGAGTCGTATT-3') (SEQ ID NO: 19) and (5'-AATACGACTCACTATAGNN-3') (SEQ ID NO: 20)] and ligase were then added to the supernatant solution and incubated at 16 °C overnight. The ligase was then heat inactivated at 65 °C for 10 minutes.

Paragraph beginning on page 32, line 3:

The samples were then amplified with PCR using T3 (5'-ATTAACCCTCACTAAAG-3') (SEQ ID NO: 21) and T7 5'-d(TAATACGACTCACTATAGGG)-3' (SEQ ID NO: 22) sequencing primers (Operon) in a 50 ml reaction containing 10⁶ copies of each target DNA, 1 unit AmpliTaq Gold polymerase (Perkin Elmer), 2 uM each primer, 200 uM dNTPs, 15 mM tris-HCl (pH 8.0), 50 mM KCl and 2.5 mM MgCl₂.